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DRAPER *et al.*
Appl. No. 09/719,002
(National Phase of International Appl. No.
PCT/GB99/01949, filed June 21, 1999)

Remarks

By the foregoing amendment, Applicants have amended the international application to place the specification into proper format for U.S. practice. In particular, Applicants have amended the specification to indicate that the international application was published in English and to direct the entry of the SEQ ID NOs identified in the sequence listing. Hence, no new matter has been added by the foregoing amendment, and entry and consideration of the same is respectfully requested.

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Conclusion

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

It is respectfully believed that this application is now in condition for examination. Early notice to this effect is earnestly solicited.

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,

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Version with markings to show changes made

The first full paragraph after the title on page 1 was replaced with the following:

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is the National Phase of International Application No. PCT/GB99/01949, filed June 21, 1999, which was published in English.

The first paragraph on page 2 (Table 1) was replaced with the following paragraph:

Table 1

| Promoter Response Elements | | | |
|----------------------------|---------------|-------------|----------------------|
| Name | Sequence | Sensitivity | |
| ABRE | CCACGTT | ABA | |
| DRE1 | TACCGACAT | Drought | |
| E-8 | ATAAGGGGTTGGT | | <u>(SEQ ID NO:5)</u> |
| G Box | GTGTCAC | | |
| H Box | GGTAGG | | |
| JA Box | CCCTATAGGG | JA? | <u>(SEQ ID NO:6)</u> |
| Myb | TGGTTA | | |
| Myc | CANNTG | | |
| PR Box | AGCCGCC | Ethylene | |
| TCA | TTATCTCCTT | | <u>(SEQ ID NO:7)</u> |

The second paragraph on page 5 was replaced with the following paragraph:

A number of elements present in PR gene promoters have been identified. The PR-2d gene (encoding a [b] β -1,3-glucanase) from tobacco is expressed in tissue undergoing hypersensitive response (HR) following tobacco mosaic virus (TMV) challenge and is induced by exogenous SA (Shah *et al.*, *Plant J.* [10] 10:1089-1101 (1996)). Region -364 to -288 in the PR-2d promoter confers SA sensitivity and a 25 bp element in this region is recognised by nuclear factors from tobacco. An SA responsive element has also been isolated from the CaMV 35S promoter at position -90 to -46. The element corresponds to an as-1 site (Qin *et al.*, *Plant Cell* [6] 6:863-874 (1994)). The sequence TCATCTTCTT (SEQ ID NO:8) is repeated several times in the barley β -1,3-glucanase promoter and is present in over 30 stress-induced genes (Goldsbrough *et al.*, *Plant J.* [3(4)] 3(4):563-571 (1993b)). This region binds 40 kDa tobacco nuclear proteins, the binding of which is increased in SA-treated plants. Buttner *et al.*, *Proc. Natl. Acad. Sci. USA* [94] 94:5961-5966 (1997) have shown that Arabidopsis ethylene responsive element binding proteins bind to the PR box and that PR- and G-boxes exhibit synergistic effects.

The third paragraph on page 28 was replaced with the following paragraph:

FIGURE 1 shows the Nucleotide sequence of AoPRT-L cDNA (SEQ ID NO:2) together with the predicted amino-acid sequence of AoPRT-L (SEQ ID NO:3). The sequences and positions of

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binding of the primers used for IPCR (SEQ ID NO:4 and SEQ ID NO:10, respectively) are indicated above the cDNA sequence and relevant enzyme restriction sites underlined.

The last paragraph on page 28 was replaced with the following paragraph:

FIGURE 6 shows the Nucleotide sequence of the AoPRT-L promoter (SEQ ID NO:1). Sequences with homology to characterised promoter elements are boxed.

The last paragraph on page 31 was replaced with the following paragraph:

P1 5'-CGCGGAATTCGGTGTAGGTGCATTTGTTGG-3' (SEQ ID NO:9) (105-86 bp) and

Eco RI

The first paragraph on page 32 was replaced with the following paragraph:

P2 5'-CGCCTGCAGCCAATCCTGGACCCTCACCG-3' (SEQ ID NO:10) (152-172 bp)

[Pst I] PstI

The last two paragraphs on page 32 were replaced with the following paragraphs:

5'- GGGTACCAAGCTTCTTATTGCGACCTGACTCTC 3' (SEQ ID NO:11)

KpnI HindIII

5'- CGCGGATCCGTCGACCTGCAGGATTGGTTGTGTGTTGTTTT 3' (SEQ ID NO:12)

BamHI Sall PstI

The second full paragraph on page 40 (Example 12) was replaced with the following paragraph:

Example 12 - Identification and multimerisation of an SA/BTH responsive element in the AoPRT-L promoter.

A series of 3 AoPRT-L 5' promoter deletion - GUS fusion constructs were constructed using the following primers designed to regions of the AoPRT-L promoter (Figure 15a):-

5' GCGAAGCTTCCATGTCATGAGAGAAGCAC 3' (-361 bp)(SEQ ID NO:13)

HindIII

5' GCGAAGCTTTTGGAAACTGAATACCTACA 3' (-247 bp)(SEQ ID NO:14)

HindIII

5' GCGAAGCTTACAAAGGCTTAGACTTTCCA 3' (-132bp)(SEQ ID NO:15)

HindIII

Each of the above primers, in conjunction with the primer below, was used in a PCR reaction with p22-JIT60 as template:-

5' GGGATCCGTCGACCTGCAGATTGGTTGTGTGTTGTTTTG 3' (SEQ ID NO:16)

BamHI Sall PstI

The second paragraph on page 41 was replaced with the following paragraph:

In order to construct an AoPRT-L promoter that has higher expression, the region -247 bp to -133 bp was amplified from p22-JIT60 and placed twice in front of a -247 bp AoPRT-L promoter. This AoPRT-Lx3 promoter was constructed as follows:- The primers below were used to PCR the 0bp to -247bp AoPRT-L promoter from p22-JIT60.

5'-TCTAGGGTACCCTTTGCGTGGTCGACTTGGAAACTGAATACCTAC-3' (SEQ ID NO:17)

KpnI

SalI

5' GGGATCCGTCGACCTGCAGATTGGTTGTGTGTTGTTTTG 3' (SEQ ID NO:16)

BamHI SalI PstI

This was cloned as a KpnI, PstI fragment into pUC19. The 133bp to-247bp pAoPRT-L region was amplified with the primers:-

5' TCTAGGGTACCCTTTGCGTGGTCGACTTGGAAACTGAATACCTAC 3' (SEQ ID NO:18)

KpnI

SalI

5' GAAAGTCTAAGCCTCGAGGGAATAAGGTACGAGTTCGTGGAC 3' (SEQ ID NO:19)

XhoI